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Bio Hydrogen Production from Pharmaceutical Waste Water Treatment by a Suspended Growth Reactor Using Environmental Anaerobic Technology

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors RHK and SVM made the plan, explained data, and dissected the results involved in the preparation of manuscript. Author RHK carried out experimental work and author AVVSS reviewed the scientific background. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Hydrogen (H₂) is considered as the future fuel. The present work on "Bio Hydrogen Production from Pharmaceutical Waste Water Treatment by a Suspended Growth Reactor Using Environmental Anaerobic Technology. This is an appreciated approach at wealth generation through value addition to wastes. The optimization process included the selection of ideal co-substrate (sucrose) and nitrogen source (DAP) to examine the feasibility of hydrogen production from industrial effluent in a 50%-50% mixture of the complex feed and the industrial effluent. Hydrogen gas produced in the reactor is estimated using a gas sensor. This equipment is a generic gas-monitoring instrument with microprocessor based electronics interfacing with std. 4 to 20 mA alarm/control systems. The inlet pH (feed) was maintained at 6 while the outlet pH monitored after detention time

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showed a slight variation (4 to 5.4) throughout the reaction periods. The variation in Volatile fatty acids (VFA) was evident up to 21 day of operation, and thereafter stabilized in and around 2600 mg/l indicating the steady state condition of the reactor. The alkalinity values variation indicated an increase in system response to acidogenic fermentation process. The variation of COD reduction (%) indicates multitude of variations as the experiment proceeds indicating perfect degradation of the organic substrate present in the culture aimed towards hydrogen production. VFA evaluation through High power liquid chromatography (HPLC) indicated presence of acetic acid within the system which could be the possible substrate for hydrogen production. During sequencing phase operation, the hydrogen values given by the experimental run with the effluent as the main substrate showed greater production rate (0.81 mmol/hr) when compared to that produced in the previous cases using only synthetic (0.086 mmol/hr) and complex feeds (0.29 mmol/hr) respectively. The described process has the dual benefit of combined H₂ production and wastewater treatment in an economical, effective and sustainable way.

Keywords: Biohydrogen; suspended growth reactor; complex feed and Pharmaceutical effluent.

ABBREVIATIONS

DAP: Diammonium Phosphate; VFA: Volatile Fatty Acids; COD: Chemical Oxygen Demand; HPLC: High Power Liquid Chromatography; HRT: Hydraulic Retention Time; BOD: Bio Chemical Oxygen Demand; TDS: Total Dissolved Solids; rpm: revolutions per minute; mv: Milli Volt; ORP: Oxidation reduction potential; DBT: Department of Biotechnology, UASB: Upflow Anaerobic Sludge Blanket Reactor; CSTR: Continuous Flow Stirred Tank Reactor; FAS: Ferrous Ammonium Sulfate; UASBR: Upflow Anaerobic Sludge Blanket Reactor; STR: Stirred Tank Reactor; AB: Acidogenic bacteria; MB: Methanogenic bacteria; VSS: Volatile Suspended Solids; MEC: Microbial Electrolysis Cell; OLR: Organic-Loading-Rate; L: liter.

1. INTRODUCTION

Hydrogen as sustainable energy carrier has garnered considerable attention due to its clean, high-energy yield and renewable nature. Recently, hydrogen production through biological approach has attracted significant interest and is deemed to be one of the emerging areas in the bio-energy research. This approach provides dual benefits where negative valued waste can be utilized for renewable energy generation along with treatment. For the past six years significant research was reported on biohydrogen (H₂) production by dark-fermentation process utilizing different types of waste/wastewater employing mixed consortia as biocatalyst. ISI Web of knowledge indexed rapid increase in publications per year from five in 2005 to 66 in 2011, which is also attracting good citations (900 citations in 2011) in this area of research [1].

The global shortage of fossil fuel and the severe environmental pollution derived from using fossil fuels have forced the developed countries in the world to search for alternative or new energy sources. Hydrogen has emerged as one of the most promising new energy carriers because it is clean, recyclable, efficient, and can be used in fuel cells to generate electricity [2]. At present, hydrogen is mainly produced from fossil fuels [3] via chemical or thermo chemical methods, while a cost-effective and pollution-free means (such as biological methods) for hydrogen production is still in great demand. Hydrogen can be generated

through natural biochemical pathways, such as photosynthesis and fermentation. In green algae or cyanobacteria, hydrogen is formed through photolysis of water, followed by an electron transfer process catalyzed by hydrogenase or nitrogenase [4-5]. Hydrogen can also be generated via fermentative conversion of organic substrates through a metabolic route that is either light-dependent (photofermentation; e.g., photosynthetic bacteria) or light-independent (dark fermentation; e.g., anaerobic bacteria) [6-9]. Early researches on biohydrogen production were mainly focused on bio-photolysis of water by algae and cyanobacteria as well as photofermentation of organic substrates by photosynthetic bacteria [10]. However, after the mid 1990s much attention has been paid to dark fermentation, in which H₂ is produced from organic compounds (especially carbohydrates) by anaerobic bacteria (e.g., acidogenic bacteria) or *Enterobacter* strains [11] with the aid of hydrogenase. The major soluble metabolites from dark fermentation include volatile fatty acids and alcohols [12].

Biohydrogen production utilizing negative valued waste through dark-fermentation process is one of the emerging areas. Reported conditions for H₂ production are significantly variable and comparative analysis of data is major problem for unified understanding [13]. Dark fermentation normally achieves a much higher H₂ production rate than water photolysis and photo-fermentation and also has the advantage of simultaneous waste reduction and clean energy (H₂) generation. Most recent studies on hydrogen fermentation with anaerobic bacteria used pure bacterial isolates as the hydrogen producer [14]. Acclimated sewage sludge or microflora was also used for hydrogen production in some cases [15-19]. Our laboratory has focused on bioreactor and biocatalyst design for anaerobic hydrogen production with municipal sewage sludge [20]. We found that to ensure a high biohydrogen production rate, it is of great importance to maintain a sufficient amount of hydrogen-producing bacterial population in the fermentor while operating at a high substrate loading rate (or a low hydraulic retention time (HRT) [21-22]. However, it is extremely difficult to achieve that in a continuous flow stirred tank reactor (CSTR) because washout of the biomass usually occurs at low HRT. Attempts to enhance biomass retention by physical or biological immobilization of cells were shown to attain better hydrogen production performance than that of CSTR, with hydrogen production rates ranging from 0.25–1.85 L/h/L [23-25]. Nevertheless, the matrices used for cell immobilization inevitably occupy significant space in the reactor, limiting cell density and possibly generating mass transfer barriers to substrates and products [26]. To avoid the problems caused by using immobilization matrices, granular sludge was generated to enhance cell retention and biomass concentration simultaneously [27] However, the feasibility of using the granular sludge system relies on the ability to control the timing and conditions of sludge granulation as well as to maintain the stability and activity of granular sludge.

Hydrogen production by electrohydrogenesis in a microbial electrolysis cell (MEC) is a new method for generating hydrogen from acetate. By using a single-chamber microbial electrolysis cell lacking a membrane, it was possible to produce hydrogen at high yields using effluent from an ethanol-type reactor for biohydrogen production. MEC achieved greater hydrogen yields and production rates than dark-fermentation as well as greater energy efficiencies. This two stage process could result in an electrical energy demand of only 1.12kWh/m³ H₂, which is much less than that needed for water electrolysis (5.6kWh/m³ H₂). However, a buffer was needed as the unamended effluent resulted in poor performance at the pH of 4.5–4.6. Methane gas was produced in low amounts at higher applied voltages, but it could not be completely eliminated. Further improvements in the process should focus on developing an acid-tolerant electrogenic community, and additional methods to limit methane generation [28].

Exploitation of wastewater as substrate for H₂ production with concurrent wastewater treatment is an attractive and effective way of tapping clean energy from renewable resources in a sustainable approach. This provides dual environmental benefits in the direction of wastewater treatment along with sustainable bioenergy (H₂) generation. However, the microbial conversion of substrate by anaerobic fermentation is a complex series of biochemical reactions manifested by diverse group of selective bacteria. However the process of hydrogen production seems to dependent on the type of wastewater and organic loading rate. Though efficient research study has been affected in this field with a high degree of success in achieving the established objectives and wastewater characteristics plays a major role in determining the stability in system performance especially in case of hydrogen production through anaerobic fermentation.

The Chemical wastewater contains complex organics, such as polysaccharides, proteins and lipids, which on hydrolysis form sugars, amino acids, and fatty acids. In subsequent acidogenic reaction, these intermediate products are converted to volatile fatty acids (VFA), which are further degraded by acetogens, forming acetate, CO₂, and H₂. Lastly, both acetate and H₂/CO₂, are converted by methanogens to CH₄. To harness H₂ as end product from anaerobic process instead of CH₄, inhibition of specific biochemical reaction (methanogenic) and enhancement for specific biochemical reaction (acidogenic) are important prerequisites. Also optimized operating conditions can result in good H₂ yield. In this direction, we have made an attempt to harvest H₂ from chemical treatment through anaerobic fermentation in suspended growth bioreactor using anaerobic mixed consortia, by restricting the methanogenic activity and manipulating operating conditions of the reactor.

Hydrogen production through anaerobic fermentation of synthetic feed was then studied in an upflow suspended film batch reactor. Synthetic feed consists of specific concentrations of several nutrients required for anaerobic fermentation. The process parameters were set depending on the optimization studies. This process aimed at establishing hydrogen production in a 1 L suspended reactor. Similar studies were performed in a suspended growth anaerobic system (stirred tank reactor) having an in-built turbine and operated by a magnetic stirrer. The hydrogen production was monitored and sequencing results were used to estimate the kinetic parameters of the reaction. The suspended growth anaerobic system was fed with optimized substrate, co-substrate and nitrogen sources along with several other nutrients, which is referred to as complex feed. This process aims at studying the variations of hydrogen production with nutrient addition. Substrate conversion efficiencies of the complex feed was studied and compared with that of the synthetic feed studied in the previous stages, to establish the degree of success of the optimization process [29].

In an effort to evolve a useful user-friendly, eco-friendly and economical process, the present study was taken up. The objective of this study was to examine the feasibility of anaerobic hydrogen production starting from a mixture of complex feed and Pharmaceutical effluent at a ratio 1:1.

2. EXPERIMENTAL DETAILS

The present study was to investigate the bio hydrogen production from Pharmaceutical waste water treatment by a suspended growth reactor using environmental anaerobic technology.

2.1 Wastewater Characteristics

The detailed characteristics of the wastewater collected from bulk drug industry (NATCO-Pharma Limited, Hyderabad, India) used in this study as feed were given in Table 1.

Table 1. Characteristics of wastewater

Parameter	Concentration
pH	7.4
Color	Yellow
TDS	16500 mg/L
COD	6080 mg/L
Sulfides	25-40 mg/L
BOD	810 mg/L
Chlorides	5538 mg/L

2.2 Anaerobic Sludge Characteristics

The sludge used in the experiment was characterized for the following parameters:

1. pH and ORP : At 1:10 dilutions , pH : 7.7 and ORP : -62.1mV.
2. VFA: At 20 dilutions VFA was found to be 2119.9 mg/ml.
3. COD: At 100 dilutions, COD was found to be 4000mg/ml.
4. Total solids: 74.25 mg / ml sludge.
5. Total Organic Carbon = 22.92.
6. Organic Matter Estimation (% carbon- 13.48; % organic matter: 23.48).

2.3 Inoculum Development for Hydrogen Production

Properly pretreated mixed anaerobic sludge for process startup was procured from a lab scale UASB reactor used in treating chemical wastewater for almost 3 years. The inoculum was subjected to heat treatment at 80°C for 24 hours followed by acid treatment at pH 3 adjusted with ortho-phosphoric acid and left undisturbed for 48 hours. Further treatment with 0.2 g/l of 2-bromethansulfonic acid sodium salt (C₂H₄BeNaO₃S) for 24 hours was performed to inhibit the methanogenic bacteria present in sludge under aseptic anaerobic conditions. A 20ml of anaerobic Innoculum was added to the anaerobic reactor in aseptic anaerobic conditions to a mixture of 33ml effluent and 22ml of sewage. The characteristics of the anaerobic inoculum was as follows

- a. Suspended Solids - 13500 mg/L.
- b. Volatile Suspended Solids - 7600 mg/L.
- c. pH (1:1 dilutions)- 6.85.

2.4 Upflow Anaerobic Sludge Blanket Reactor (UASBR) Start Up and Reactor Operation

The reactor was inoculated with biomass acquired from an operating laboratory scale upflow anaerobic sludge blanket reactor (UASBR) unit, which has been in operation continuously for 3 years for the treatment of complex chemical effluents. About 300ml of the anaerobic sludge (VSS: 3.5 g/L) from the anaerobic reactor was acquired and fed to the suspended reactor. It

was subjected to acid treatment at pH 3 adjusted with ortho-phosphoric acid and left undisturbed for 48 hours. Further treatment with 0.2 g/L of 2-bromethansulfonic acid sodium salt ($C_2H_4BeNaO_3S$) for 24 hours was performed to inhibit the methanogenic bacteria present in sludge under aseptic anaerobic conditions.

The reactor has a total working volume of 1.3 L capacity. The hydrogen fermentation was conducted at mesophilic temperature ($29 \pm 2^\circ C$). The pH was maintained at 6 to ensure that the fermentation process does not yield a drastic drop in the pH value after a HRT of 24 hours. This decision was based on the optimization studies [30]. The suspended reactor was started with synthetic feed. About 1 L synthetic feed was taken and fed to the reactor and the inlet and outlet samples (after a HRT of 24 hours) were collected and was continuously examined for pH, ORP, VFA, COD and hydrogen gas production. The suspension was maintained by recalculating the feed through a tube aided by a peristaltic pump operating at 100 rpm. Initial 5 days of operation in up flow feed recirculation mode produced negative results due to absence of suspension. The next 10 days operation was performed by up flow of sludge through the recirculation tube to keep the reactor in suspension. Then, sequencing was done at the HRT intervals of 1, 2, 4, 6, 8, 10, 12, 24 and 48 Hours of incubation. [29].

2.5 Hydrogen Production in a Stirred Tank Reactor (STR) Maintained under Suspension

The inoculum from the (UASBR) suspended reactor was directly transferred to a stirred tank reactor (STR) fitted with a 2-blade axial turbine consisting of a magnetic pellet that can be operated with the help of a magnetic stirrer. This reactor maintained a suspension by the movement of the turbine blades, which stirred the microbial culture to move in the working volume in an irregular manner.

2.6 Stirred Tank Reactor (STR) Configuration

The stirred tank reactor, manufactured by Nalgene (Germany), consists of a plastic vessel with a curved bottom. The reactor has a magnetic pellet at the center of a 2 axial blade turbine, which rotates about its axis with the help of magnetic force developed by a magnetic stirrer.



Anaerobic batch stirred tank reactor

The reactor has two openings at the top for inlet and outlet purposes. The various design details of the reactor are:

Total Capacity: 2.2 L.
 Working Capacity: 1.25 L.
 Overall height: 266 mm.
 Outer Diameter of the reactor: 137 mm.

2.7 Reactor Start Up

This reactor did not have a startup procedure because the inoculum was taken directly from the upflow anaerobic sludge blanket reactor (UASBR), which was recently treated to inhibit methanogenesis.

2.7.1 Reactor setup and inlet conditions

The reactor has a total working volume of 1.25 L capacity. The hydrogen fermentation was conducted at mesophilic temperature ($29 \pm 2^\circ\text{C}$). The pH was maintained at 6 to ensure that the fermentation process does not yield a drastic drop in the pH value after a HRT of 24 hours. This decision was based on the optimization studies. The suspension was maintained by the movement of turbine blades powered by a magnetic stirrer operating at 100 rpm.

2.7.2 Synthetic feed studies-Reactor operation

The reactor was started with synthetic feed, which has the composition as shown in Table 2. About 1 L of synthetic feed was taken and fed to the reactor and the inlet and outlet samples (after a HRT of 24 hours) were collected and was continuously examined for pH, ORP, VFA, COD and hydrogen gas production. The reactor was analyzed for the various important process parameters for the inlet and outlet samples for around 13 days. After a steady state was attained, the sequencing at the HRT intervals of 1, 2, 4, 6, 8, 10, 12, 24 and 48 Hours of incubation. The samples were regularly monitored for pH, VFA, Alkalinity, COD, Glucose, VSS and Hydrogen gas parameters. HPLC for the samples was carried out. The reactor kinetics and substrate conversion efficiency was also calculated using the biomass and substrate concentrations at various time intervals in sequencing period [29].

Table 2. Synthetic feed composition

Nutrients	Composition (g/L)
NH ₄ Cl	0.5
KH ₂ PO ₄	0.25
K ₂ HPO ₄	0.25
MgCl ₂ .6H ₂ O	0.3
FeCl ₃	0.025
NiSO ₄	0.016
CoCl ₂	0.025
ZnCl ₂	0.0115
CuCl ₂	0.0105
CaCl ₂	0.005
MnCl ₂	0.015
(C ₆ H ₁₂ O ₆)	3

2.7.3 Complex feed studies -reactor operation

Complex feed refers to the variable concentrations of nutrients required to enhance fermentation and hydrogen production process. Based on optimization studies, the complex feed was specified as shown in the Table 3. The sucrose concentration was calculated to maintain an organic loading rate of approximately 5000 mg/l. DAP concentration was based on N: P ratio of 5:1. About 1 L of the feed was taken and fed to the reactor and the inlet and outlet samples (after a HRT of 24 hours) were collected and was continuously examined for pH, ORP, VFA, COD and hydrogen gas production. The reactor was analyzed for the various important process parameters for the inlet and outlet samples for around 3 days. After a steady state was attained, the sequencing at the HRT intervals of 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours of incubation. The sequencing samples were monitored for pH, VFA, Alkalinity, COD, Sucrose and Hydrogen gas parameters. HPLC for the samples was carried out. The substrate conversion efficiency was also calculated at various time intervals in sequencing period [29].

Table 3. Complex feed composition

Nutrients	Composition (g/L)
(NH ₄) ₂ HPO ₄	0.5
MgCl ₂ .6H ₂ O	0.3
FeCl ₃	0.025
NiSO ₄	0.016
CoCl ₂	0.025
ZnCl ₂	0.0115
CuCl ₂	0.0105
CaCl ₂	0.005
MnCl ₂	0.015
(C ₁₁ H ₂₂ O ₁₁)	3.74

2.8 Fermentation of Complex Feed with Industrial Effluent

2.8.1 Reactor operation

The sucrose concentration was calculated to maintain an organic loading rate of approximately 5000 mg/L along with the industrial effluent. About 1 L of the feed containing 50% complex feed and 50% Industrial effluent was taken and fed to the reactor and the inlet and outlet samples (after a HRT of 24 hours) were collected and was continuously examined for pH, ORP, VFA, COD and hydrogen gas production. The reactor was analyzed for the various important process parameters for the inlet and outlet samples for around 3 days. After a steady state was attained, the sequencing at the HRT intervals of 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours of incubation. The sequencing samples were monitored for pH, VFA, Alkalinity, COD, Sucrose and Hydrogen gas parameters. HPLC for the samples was carried out. The substrate conversion efficiency was also calculated at various time intervals in sequencing period [29].

2.9 Analytical Procedures

The performance of reactor with complex chemical effluents was assessed by monitoring carbon removal (COD) throughout the reactor operations and during the cycle period. In addition, pH, oxidation-reduction potential (ORP), VFA, Alkalinity and suspended solids (SS)

were determined during reactor operation to assess the performance of the reactor. The analytical procedures for monitoring the above parameters were adopted from the procedure outline in the Standard methods. The method performed for determination of physicochemical parameters was adopted from standard methods of American public health association [31].

2.9.1 Hydrogen gas estimation

Hydrogen gas produced in the reactor is estimated using a gas sensor, FMK satellite 4-20 mA version (ATMIGmBH Inc.). This equipment is a generic gas-monitoring instrument with microprocessor based electronics interfacing with std. 4 to 20 mA alarm/control systems. Target gas and measuring range depend on type of sensor chosen. The electrochemical sensors designed for use with the FMK satellite feature an integrated data memory. When a new sensor is fitted, the instrument's electronics will load operating parameters of the sensor into microprocessor's memory. The current flowing through the sensor is amplified electronically, digitized and temperature compensated and resulting concentration value is given as an analog 4 to 20 mA output signal. This output signal usually displays the %volume of hydrogen in the reactor air space. This is converted to mmol using the calculations as explained below.

By Ideal Gas Law,

$$P * V = N * R * T$$

Where, P = pressure of hydrogen in the reactor = 1 atm

V = volume of hydrogen in the reactor (ml)

N = number of moles of hydrogen (mol)

T = temperature = 27°C = 300 K

R = Ideal Gas Constant = 0.0821 L x atm/mol x K

$$\rightarrow 1 \times V = N \times 0.0821 \times 300$$

$$\rightarrow V = N \times 24.63 \text{ L} = \text{Volume of hydrogen}$$

Now, % vol = (volume of hydrogen/volume of air) x 100 = XC (assumption)

Where XC is assuming percentage of hydrogen per volume

$$\rightarrow \text{Volume of hydrogen} = 0.01 \times XC \times \text{Volume of air}$$

$$\rightarrow N \times 24.63 = 0.01 \times XC \times \text{Volume of air}$$

$$\rightarrow \mathbf{N = \text{number of moles of hydrogen} = 4.06 \times 10^{-4} \times \text{Volume of air} \times XC}$$

For the upflow suspended reactor,

Volume of air = 300 ml = 0.3 L.

$$\rightarrow \mathbf{N = 0.122 \times (\%vol) \text{ mmol}}$$

For anaerobic contact stirred reactor,

Volume of air = 850 ml = 0.85 L.

$$\rightarrow \mathbf{N = 0.3451 \times (\%vol) \text{ mmol.}}$$

3. RESULTS AND DISCUSSION

The anaerobic stirred tank reactor showed consistency in its results on feeding with the synthetic feed. This set of experiments aims at studying the variation of process parameters on using the optimized co-substrate and nitrogen source as the feed for the reactor. The complex feed containing sucrose and DAP was calculated to maintain a COD value of 5000 mg/L. The sequencing procedure also showed a number of variations in the process parameters especially the hydrogen production rate. However, the hydrogen values estimated

from the anaerobic fermentation of the complex feed was greater than that estimated with the synthetic feed indicating the success of the optimization studies [29]. The objective of the present research is up scaling and Sequencing studies with anaerobic suspended growth reactor for Bio hydrogen production using complex feed and Pharmaceutical Effluent (1:1) ratio.

3.1 Up Scaling with Anaerobic Suspended Growth Reactor Using Complex Feed and Pharmaceutical Effluent (1:1)

The basic aim to maintain reactor-operating environment at pH 6.0 was to facilitate the inhibition of MB at the same time to create conducive environment for effective functioning of Acidogenic bacteria (AB). The most effective way to enhance H₂ production from the anaerobic culture is to restrict or terminate the methanogenesis process by allowing H₂ to become an end product in the metabolic flow. In this study, it was observed that adoption of low operating pH inhibited Methanogenic bacteria (MB). This facilitated generation of H₂ as terminal product of anaerobic fermentation due to suppression of the formation of CH₄. The adopted HRT of 24 h further helped to control the methanogenic reaction. Sequencing batch operation mode of the reactor used might also have influenced the H₂ evolution. The sequencing/periodic discontinuous batch mode operation facilitates controlled unsteady-state conditions and exposure time, frequency of exposure and substrate concentration can be set independent of inflow condition. This facilitated the microorganisms to periodically expose to defined process conditions and helped to select organisms that were generally more robust and able to withstand shock loads [32].

The optimization process included the selection of ideal co-substrate (sucrose) and nitrogen source (DAP) to examine the feasibility of hydrogen production from industrial effluent in a 50%-50% mixture of the complex feed and the industrial effluent. The pH values remained very low at the end of the experimental period indicating the decrease in system's performance in acidogenic fermentation process. The decrease in pH gives a favorable acid formation but it was studied that the production of hydrogen was affected and terminated by low pH. The optimum pH range for hydrogen production being in the range of 5-6 [33]. In this study, the inlet pH (feed) was maintained at 6 while the outlet pH monitored after detention time showed a slight variation (4 to 5.4) throughout the reaction periods in (Fig 1.). However, the pH was low indicating similar performance as discussed above.

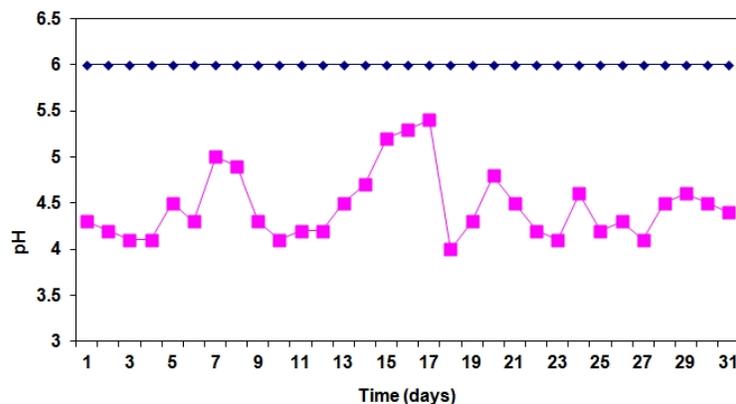


Fig. 1. Study state condition of pH inlet and outlet in a suspended growth reactor

The inlet VFA as in (Fig. 2), varied in the range of 2000 ± 500 mg/L while the outlet VFA concentration showed a significant variation from 1800 mg/L to 3400 mg/L. The variation in VFA was evident upto 21 day of operation, and thereafter stabilized in and around 2600 mg/l indicating the steady state condition of the reactor. Alternate decrease and increase in the VFA values during initial phase of reactor operation indicating a sort of instability present initially in the system.

Alkalinity plays a vital role in restricting the organic acid accumulation leading to a balanced pH level within the reactor to enable hydrogen production coupled with substrate removal during the reaction. Alkalinity during the bioreactor operation was monitored to understand the buffering activity of the reactor system [34-35]. The variation of alkalinity lies within the range of 1000 mg/L to 4400 mg/L, which is considered to be on higher side. However, the variation in alkalinity figures of the outlet recorded after 25 days showed stabilized values. The alkalinity values variation indicated an increase in system response to acidogenic fermentation process.

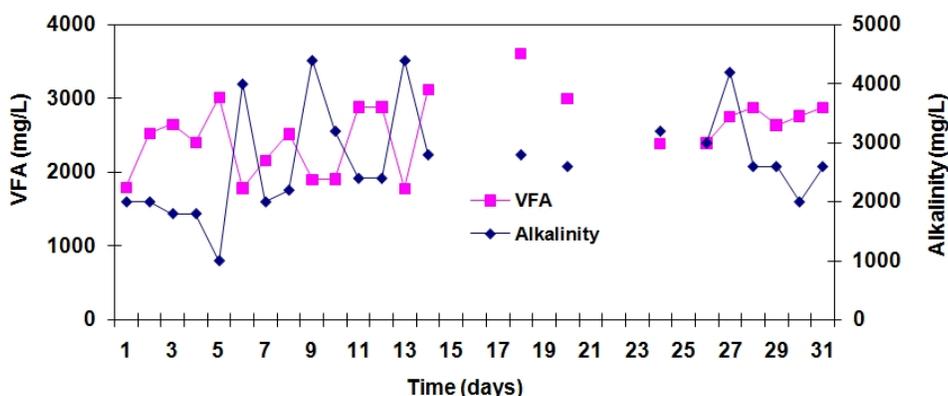


Fig. 2. Variation of VFA-concentration and alkalinity of inlet and outlet of industrial effluent feed at different HRTs

The variation of COD reduction (%) indicates multitude of variations as the experiment proceeds indicating perfect degradation of the organic substrate present in the culture aimed towards hydrogen production. This set of experimentation was continued till the system attained maximum hydrogen production on a steady scale and further was stepped up by increasing the Pharmaceutical wastewater composition in the feed.

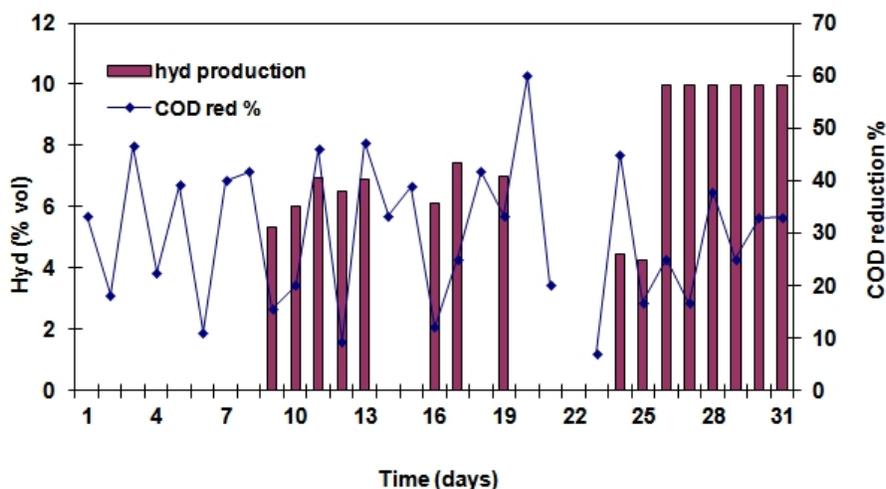


Fig. 3. Variation of COD reduction % and hydrogenproduction with different HRTs

The hydrogen production rate was found to increase after the seventh day followed by a gradual increase till the end as shown in (Fig. 3) However a decrease in the production till 25th day which was supported by decreased COD reduction followed by maximum hydrogen production till the end. This proves advantageous for understanding the feasibility of the effluent towards hydrogen production. Conducting a sequencing procedure, which is discussed in the later part of this section, the experiment was continued by increasing the concentration of Pharmaceutical effluent.

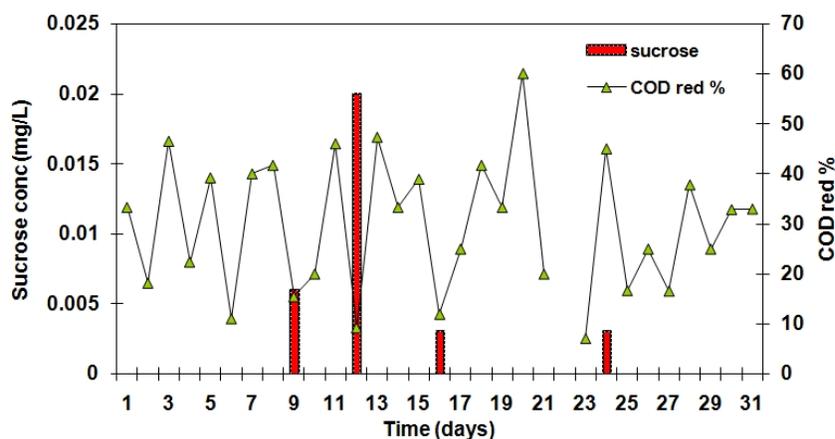


Fig. 4. variation of sucrose concentration at different HRTs with COD % reduction

Fig. 4. Variation of sucrose concentration at different HRTs with COD % reduction

Sucrose concentration was estimated at different intervals to verify the performance of the reactor is shown in (Fig. 4.) As observed from the graph the residual concentrations of sucrose were found to decrease with minimum concentration remaining at the end

representing complete utilization of the substrate and maximum hydrogen production at the end.

Monocarboxylic acids like acetic acid, Propionic acid, butyric acid, etc; and polycarboxylic acids like lactic acid, succinic acid, etc are known as volatile fatty acids (VFA). These acids under anaerobic conditions decompose to give carbon dioxide and methane. If methanogenic bacteria are inhibited and the process of decomposition is controlled at acidogenesis hydrogen gas is produced. In this study VFA evaluation through HPLC indicated presence of acetic acid within the system which could be the possible substrate for hydrogen production as in (Fig. 5.)

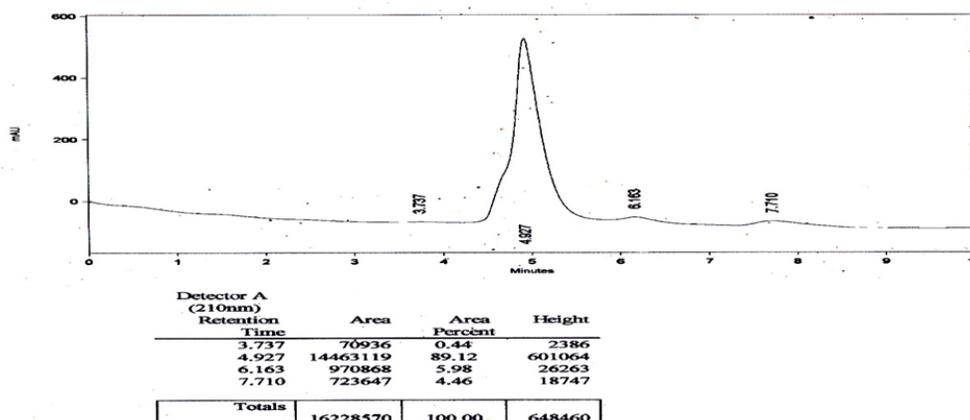


Fig. 5. VFA- Evaluation through high power liquid chromatography

3.2 Sequencing Study with Anaerobic Suspended Growth Reactor Using Complex Feed and Industrial Effluent (1:1)

Parameters such as VFA (represented as the total of all acids generated during acidogenic fermentation step), pH and alkalinity were also monitored along with COD during the cycle operation of the reactor. H₂ production is generally accompanied by acid and solvent production due to acidogenic metabolism. Generation of the acidic intermediates causes changes in the metabolic pathway of the microorganisms involved, and provides a better knowledge, which can be used to improve the conditions favorable for H₂ production. The pH drop in the bioreactor system especially in anaerobic microenvironment was considered as an index of VFA with the existing buffering capacity (alkalinity) of the system [36-37]. VFA production was always associated with conversion of organic fraction to acid intermediates in the anaerobic microenvironment with the help of specific group of bacteria. H₂ was the important product released along with VFA during anaerobic fermentation of wastewater during acidogenic phase. Acidogens grow relatively faster and are less sensitive to pH variation than acetogens/methanogens. This usually results in the accumulation of organic acids and lowering of pH, leading to the suppression of methanogenic activities, and in some cases, even process failure [38].

During sequencing phase operation, analysis of VFA, alkalinity and COD reduction was also done as shown in (Figs. 6 and 7). The variation of VFA and alkalinity during the sequencing period proves to be in acceptance with the reviewed discussions. The VFA values increased

throughout the first 12 hours registering a maximum value of 2723 mg/L at the end of the 12th hour. The VFA values then dipped to 2542 mg/L at the end of 24th hour. The Alkalinity values also decreased to 0 mg/L at the end of 2nd hour followed by a stationary value of 0 mg/L till the end of 12th hour after which it increased to 120 mg/L at the end of sequencing period. This indicates a healthy acidogenic fermentation of the substrate.

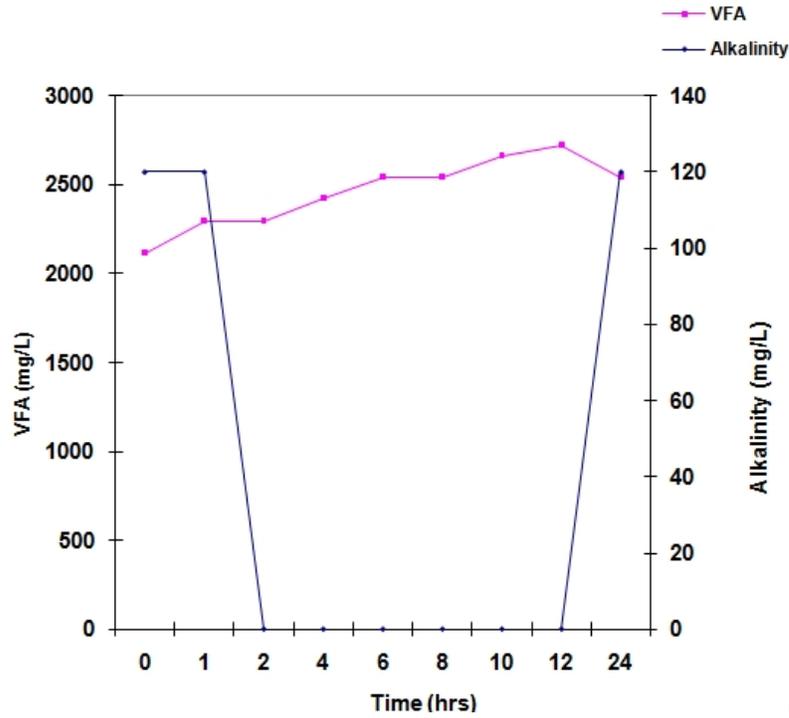


Fig.6. The variation of VFA and alkalinity during the sequencing period

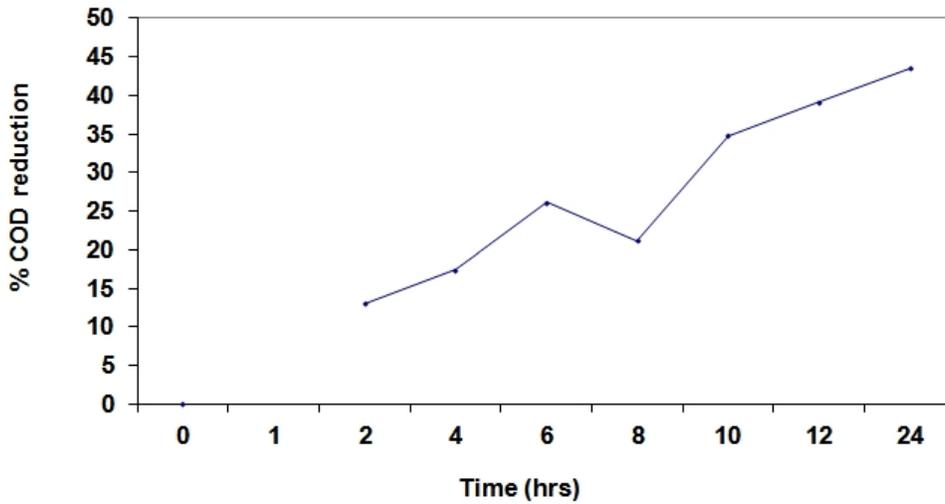


Fig. 7. The variation of COD during the sequencing period

The hydrogen production rate increased till the end of 1st hour where it registered a maximum value of 0.81 mmol/hr. Then, the values started to decrease till the 2nd hour followed by an increase till the 10th hour where it showed a value of 0.7 mmol/hr as shown in (Fig. 8.) This value was followed by a decrease till the end of sequencing period. The hydrogen values given by the experimental run with the effluent as the main substrate showed greater production rate when compared to that produced in the first two cases using only synthetic and complex feeds respectively.

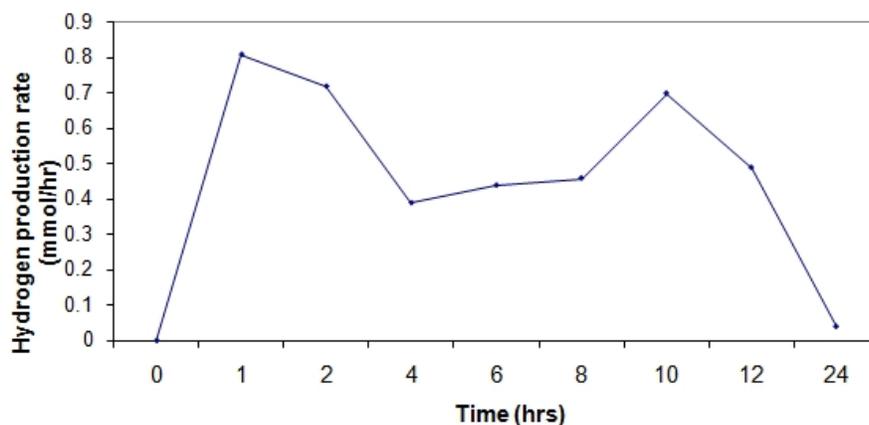


Fig. 8. Hydrogen production rate during sequencing period

The anaerobic suspended batch reactor was supplied with the pretreated anaerobic mixed culture and was fed with synthetic feed containing glucose and complex feed containing (sucrose) as co-substrates and NH₄Cl as the nitrogen source. The present research is up scaling and Sequencing studies with anaerobic suspended growth reactor for Bio hydrogen production using complex feed and Pharmaceutical Effluent (1:1) ratio. The experimental data was consolidated and depicted in Table 4.

Table 4. Details of hydrogen production from the experimental variations studied

S.No	Industrial wastewater	Organic-loading-rate (OLR)(Kg COD/cum-day)	Hydrogen production (mmol/day)
1	Designed synthetic wastewater	3.80	0.486
2	Designed Complex wastewater	4.67	3.45
3	Complex feed and Pharmaceutical Effluent (1:1) ratio	5.90	2.95

It is evident from the data that the suspended growth configuration has yielded hydrogen production without process inhibition; however the process of hydrogen production seems to dependent on the type of wastewater and organic loading rate. Though efficient research study has been affected in this field with a high degree of success in achieving the established objectives and wastewater characteristics plays a major role in determining the stability in

system performance especially in case of hydrogen production through anaerobic fermentation.

4. CONCLUSION

The study demonstrated the feasibility of H₂ generation from Pharmaceutical wastewater treatment by anaerobic fermentation in suspended growth bioreactor using anaerobic mixed inoculum. However, the process of H₂ generation was found to be dependent on the Organic-loading-rate (OLR) applied. The pretreatment steps adopted for enumerating the H₂ production from anaerobic inoculum were found to be effective. The selected reactor operating conditions (acidophilic pH 6) were found to be optimum for effective H₂ yield. Integration of suspended configuration with sequencing/ periodic discontinuous batch operation was found to be highly flexible, and has a great potential to provide the possibilities of influencing the microbial system by selectively enriching the specific group of microflora. The system is comparatively easy to operate and cost efficient. Using mixed microbial cultures is considered to be a practical, cost-effective and promising approach to achieve H₂ production in large scale. The described process has the dual benefit of combined H₂ production and wastewater treatment in an economical, effective and sustainable way. Extensive research work was in progress regarding bioprocess monitoring during hydrogen production, reactor configuration optimization, characterization of hydrogen producing bacteria, bioaugmentation strategy, etc. in the direction of biohydrogen production from Pharmaceutical wastewater using Environmental Anaerobic Technology.

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COMPETING INTERESTS

The authors declare that have no competing interests.

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